Early Therapeutic Discovery in Oncology and Beyond: Challenges and Opportunities

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Scientific Director, National Center for Advancing Translational Sciences (NCATS), National Institutes of Health (NIH)

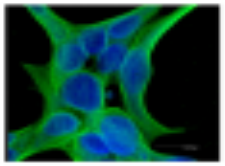
TRACO Lecture
September 30, 2019

Therapeutic discovery and development

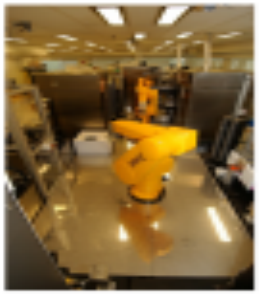
Therapeutic discovery and development: the simplistic view and the reality

Identify disease mechanism → Create testing system → Test chemicals in system → Modify (chemistry) for human use → Test in animals → Test in humans

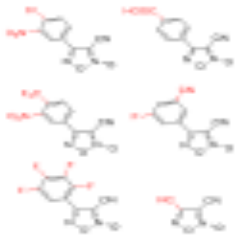
Basic Research



Assay Development



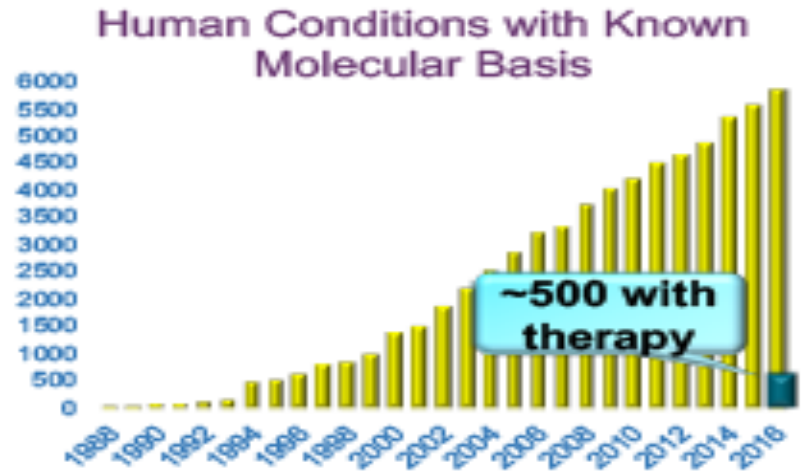
Screening

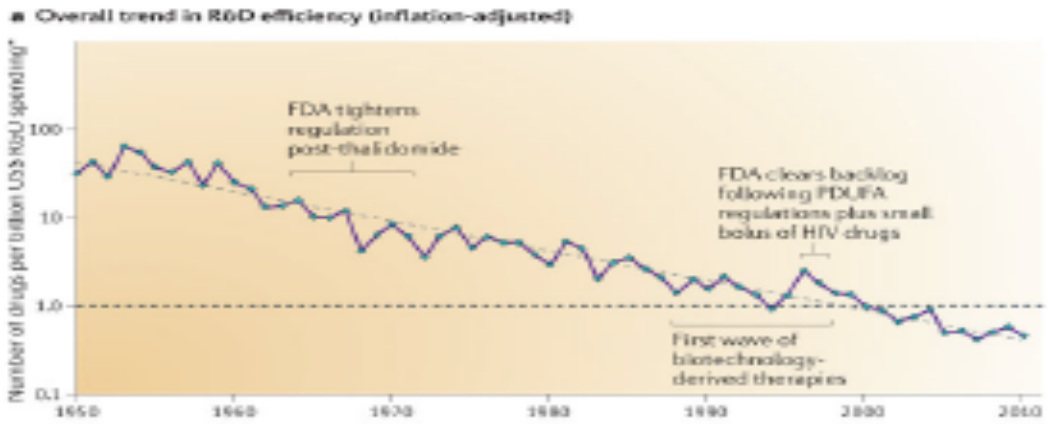


Medicinal Chemistry

Preclinical Development

Clinical Development





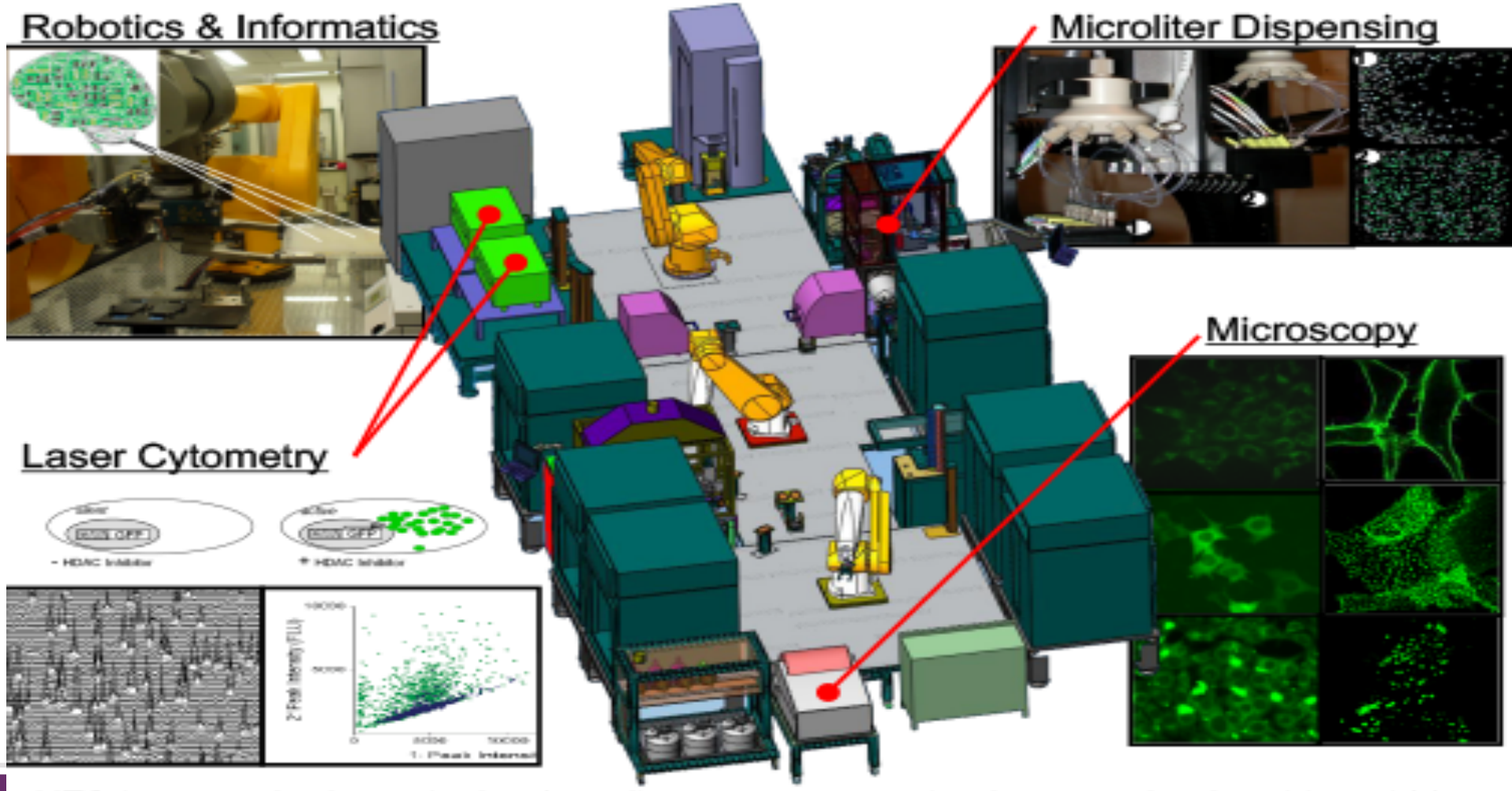
Nat Rev Drug Disc 11, 191 (2012)

Translational inefficiency

The common causes of translational inefficiency are NCATS' focus

- Predicting safety and effectiveness of new drugs
- Scalable approaches to the >6000 untreatable diseases
- Data interoperability
- Biomarker qualification process
- Clinical trial networks
- Patient recruitment
- Electronic Health Records for research
- Harmonized IRBs
- Clinical diagnostic criteria
- Clinical outcome criteria (e.g., PROs)
- Adaptive clinical trial designs
- Shortening time of intervention adoption
- Methods to better measure impact on health (or lack thereof)
- Cross-sector collaborative structures
- Translational education/workforce development

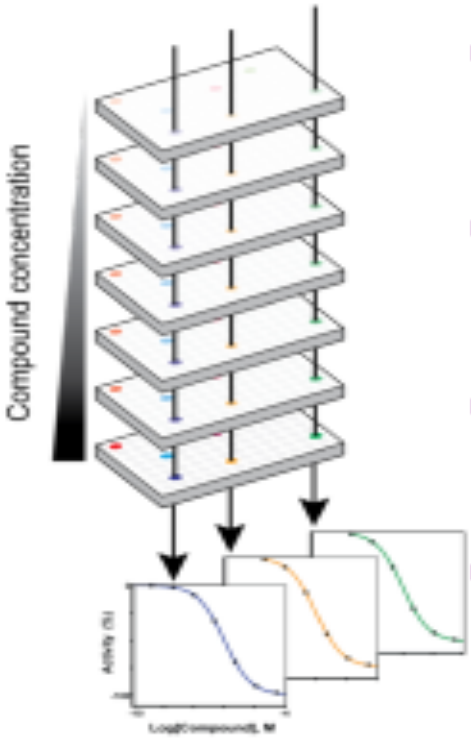
High throughput screening



HTS is a standard step in the drug discovery process but has remained problem-ridden.

Quantitative high throughput screening

Improving the Process of Early Discovery: Quantitative High-Throughput Screening (qHTS)



- Conventional screening done at one concentration
  - Not appropriate for potency testing – "dose makes the poison"
- qHTS tests compounds assayed at multiple concentrations (range: 4 logs)
- Enabled by miniaturized assay volumes (2-8 µL per test) and informatics pipeline
- Generates *pharmacological actives* instead of statistical "hits"
  - Dramatically increases reliability
  - Dramatically reduces false positives and false negatives

PNAS 103:11473

- *To date, several hundred million datapoints from several hundred screens have been generated and deposited in the public domain.*

Full integration of medicinal chemistry

Full integration of medicinal chemistry to make more effective and less toxic tool molecules and drug candidates

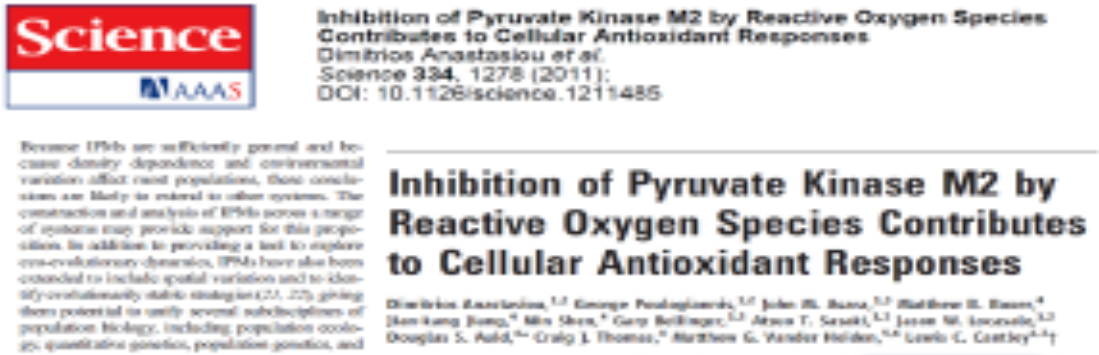
Multi-parameter optimization (potency, solubility, metabolic stability)

~200 high-quality small molecule probes/tools to study novel therapeutic concepts and to shine light on the "dark matter of the genome" have been developed and made publicly available. Some of these are being further developed as drugs.

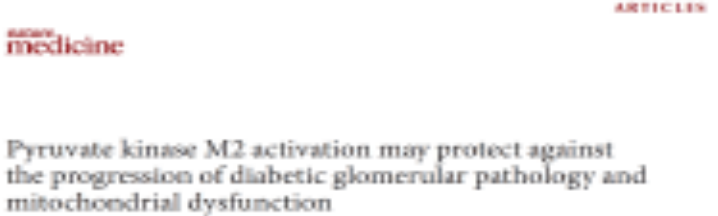
Small molecules

Example: small molecule probes to study cancer metabolism…with additional applications

- Tumors exhibit unique nutritional requirements. For example, pyruvate kinase M2 (PKM2) has been implicated in the Warburg Effect.
- A screen of PKM2, followed by medicinal chemistry optimization, produced several distinct molecules able to significantly activate the enzyme.
- These tool molecules have been used by multiple labs worldwide to study cancer biology and a subset are being further developed as therapies.



A recent development: PKM2 activation may be a way to modulate metabolism in pancreatic beta cells to manage diabetes:



Irreversible inhibition

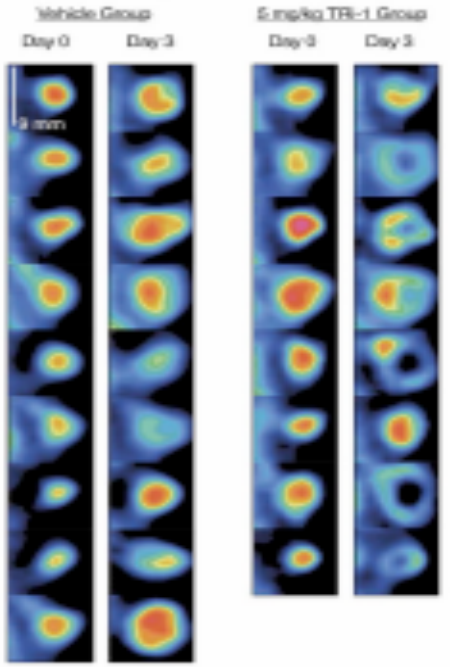
SCIENCE TRANSLATIONAL MEDICINE | RESEARCH ARTICLE

CANCER

Irreversible inhibition of cytosolic thioredoxin reductase 1 as a mechanistic basis for anticancer therapy

William C. Stafford,[1,2] Xiaoxiao Peng,[1*] Maria Hägg Olofsson,[3†] Xiaonan Zhang,[3] Diane K. Luci,[4] Li Lu,[5] Qing Cheng,[1] Lionel Trésaugues,[6†] Thomas S. Dexheimer,[4§] Nathan P. Coussens,[4¶] Martin Augsten,[3] Hanna-Stina Martinsson Ahlzén,[1††] Owe Orwar,[2,7] Arne Östman,[3,8] Sharon Stone-Elander,[9,10] David J. Maloney,[4‡‡] Ajit Jadhav,[4] Anton Simeonov,[4] Stig Linder,[3,11] Elias S. J. Arnér[1§§]

- Thioredoxin reductase (TrxR): an underappreciated cancer target involved in control of redox balance.
- TrxR1 inhibitor, discovered from a robotic screen, acts by a covalent mechanism, yet is devoid of broad reactivity against similar targets (glutathione reductase, others).
- Unique selenocysteine residue in the enzyme active site responsible for the inhibitor's reactivity (*i.e.*, covalent action highly context-dependent).
- The inhibitor is presently being developed by a new company spun out of Karolinska Institutet.



FaDu xenograft, [^{18}F]-FDG uptake

Example

Example: deep profiling of resistance development leads to an effective countermeasure

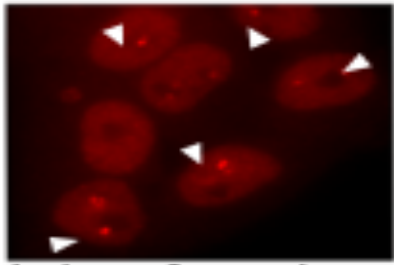
- Adaptive resistance in FMS-like receptor tyrosine kinase (FLT3)–mutant acute myeloid leukemia (AML) develops upon treatment with FLT3 inhibitors.
- To tackle the problem, the team examined the integrative in-cell kinase and gene regulatory network responses in tumors treated with FLT3 inhibitor.
- Innate immune pathway activation via the interleukin-1 receptor–associated kinase 1 and 4 (IRAK1/4) complex was found to contribute to adaptive resistance in FLT3-mutant AML cells.

Targeting cancer metastasis

The other side of the "dark genome": targeting cancer metastasis as a translational challenge

Translational problem: 90% of cancer deaths are the consequence of metastatic spread, yet *there are no drugs labeled as antimetastatic agents*

The mechanisms of metastatic transformation and spread are not well elucidated
⇒ **how do you develop therapies if you don't know the targets?**



PNC & Metastasis

The Perinucleolar Compartment (PNC) is a nuclear organelle that appears in transformed cancer cells.

PNC prevalence correlates with breast cancer, colorectal cancer, and ovarian cancer disease progression, reaching nearly 100% in distant metastases.

PNCs are composed of a complex of proteins and RNA ⇒ **no obvious single drug target**

Approach: phenotypic screen for PNC disrupters as a therapeutic approach to block metastasis

Collaborative approach

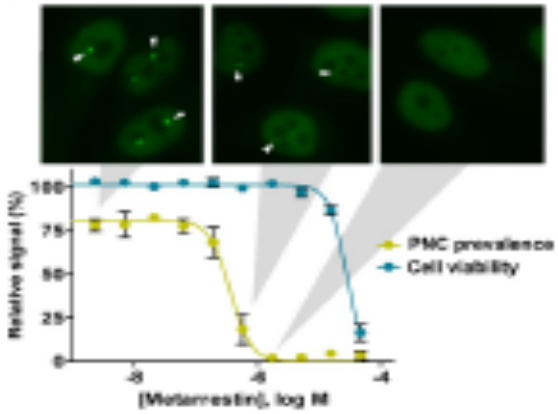
Disease expert:	Sui Huang, Northwestern University
Medicinal Chemistry:	Jeffrey Aube, University of Kansas
Clinical expert:	Udo Rudloff, NCI

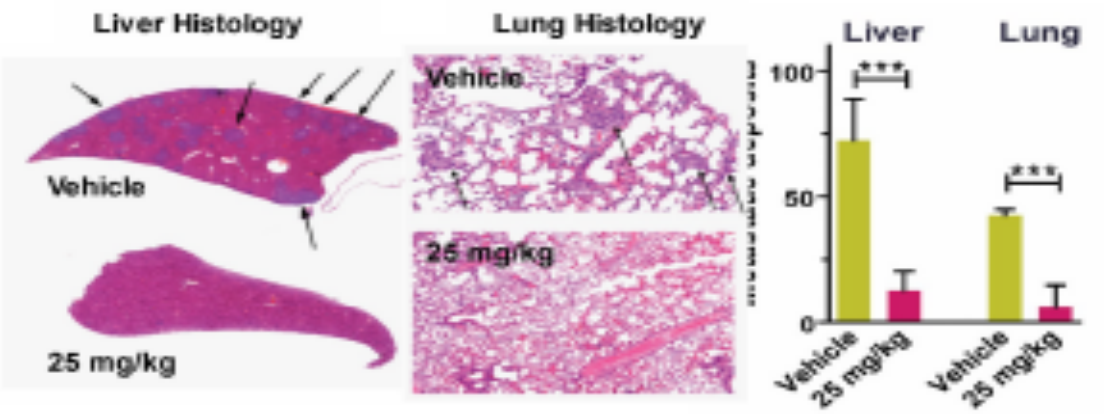
Metarrestin

Discovery of Metarrestin

Through an unbiased phenotypic screen, the team identified a class of compounds that disrupt PNC in a metastatic tumor cell line (PC3-M) without cytotoxic effects.

A chemically-optimized molecule (metarrestin) demonstrated benefit in a mouse orthotopic model of pancreatic cancer metastasis.





Sci Transl Med. 2018 May 16;10(441)

Combination screening

Combination screening to overcome drug resistance in bacteria

Collaborators: Wei Zheng (NCATS), Peter Williamson (NIAID), Karen Frank (NIH Clinical Center)

- Developed assay and used qHTS to rapidly screen 5,170 approved drugs and drug candidates against multidrug-resistant (MDR) organisms.
- Tested thousands of two-drug combinations, identified synergistic pairs.

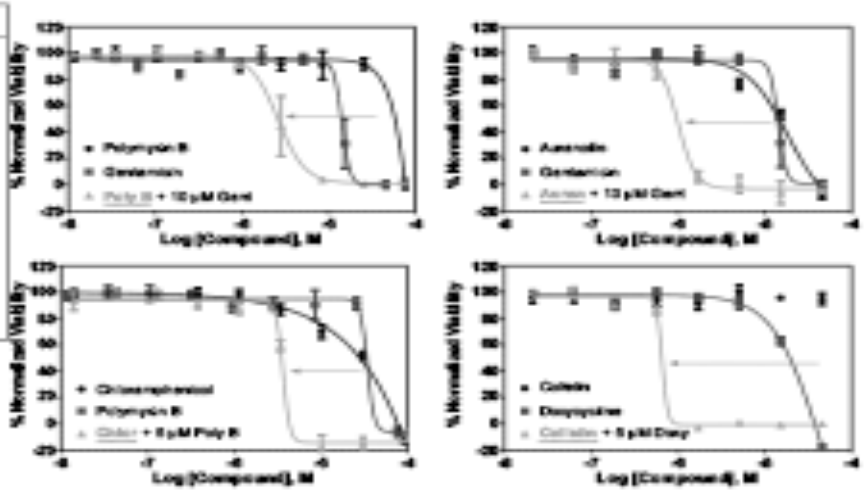
OPEN

ORIGINAL ARTICLE

Rapid antimicrobial susceptibility test for identification of new therapeutics and drug combinations against multidrug-resistant bacteria

Wei Sun[1,*], Rebecca A Wickenhauser[1,*], Miao Xu[1,*], Ned Southall[1], Sheng Dai[1], Paul Shinn[1], Philip E Sanderson[1], Peter R Williamson[2], Karen M Frank[2] and Wei Zheng[1]

"The results demonstrate this new assay has potential as a real-time method to identify new drugs and effective drug combinations to combat severe clinical infections with MDR organisms."



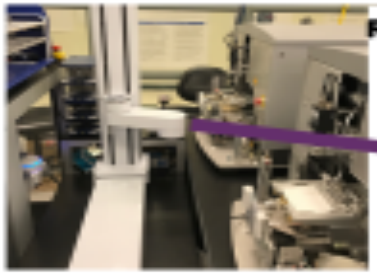
Metarrestin program

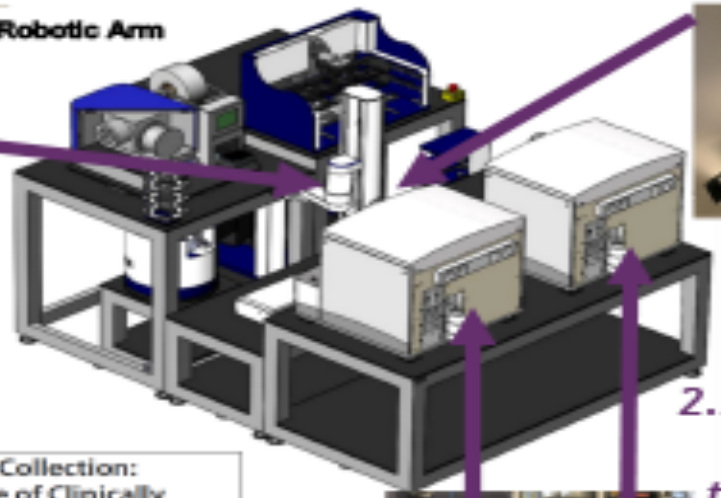
Metarrestin Program: Status and Next Steps

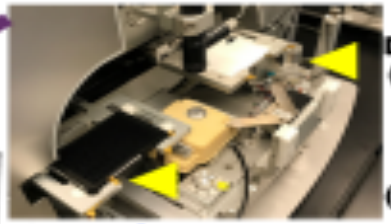
- Metarrestin is in pre-clinical development to target metastasis in pancreatic cancer.
- Pre-clinical development is currently in final stages through NCATS BrIDGs program (GMP manufacture & IND-enabling toxicity studies).
- Clinical support through NCI, goal is an IND filing in late 2019.

Drug combinations

Translation Challenge: Rapid Discovery of Drug Combinations







1. Appropriate libraries

2. Automation/ screening technologies

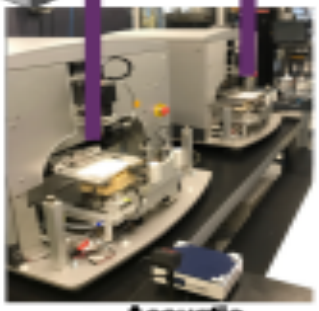
3. Informatics platform

The NCGC Pharmaceutical Collection: A Comprehensive Resource of Clinically Approved Drugs Enabling Repurposing and Chemical Genomics

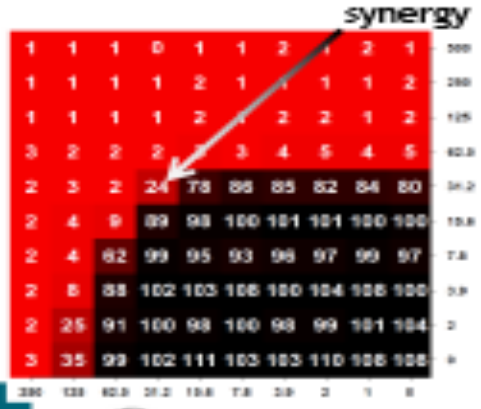
Ruili Huang, Noel Southall, Yuhong Wang, Adam Yasgar, Paul Shinn, Ajit Jadhav, Dac-Trung Nguyen, Christopher P. Austin

Small molecule compounds approved for use as drugs may be "repurposed" for new indications and studied to determine the mechanisms of their beneficial and adverse effects. A comprehensive collection of all small-molecule drugs approved for human use would be invaluable for systematic repurposing across human diseases, particularly for rare and neglected diseases, for which the cost and time required for development of a new chemical entity are often prohibitive. Previous efforts to build such a comprehensive collection have been limited by the complexities, redundancies, and semantic inconsistencies of drug naming within and among regulatory agencies worldwide; a lack of clear conceptualization of what constitutes a drug; and a lack of access to physical samples. We report here the creation of a definitive, complete, and non-redundant list of all approved molecular entities as a freely available electronic resource and a physical collection of small molecules accessible to high-throughput screening.

www.ScienceTranslationalMedicine.org 27 April 2011 Vol 3 Issue 80 80ps16

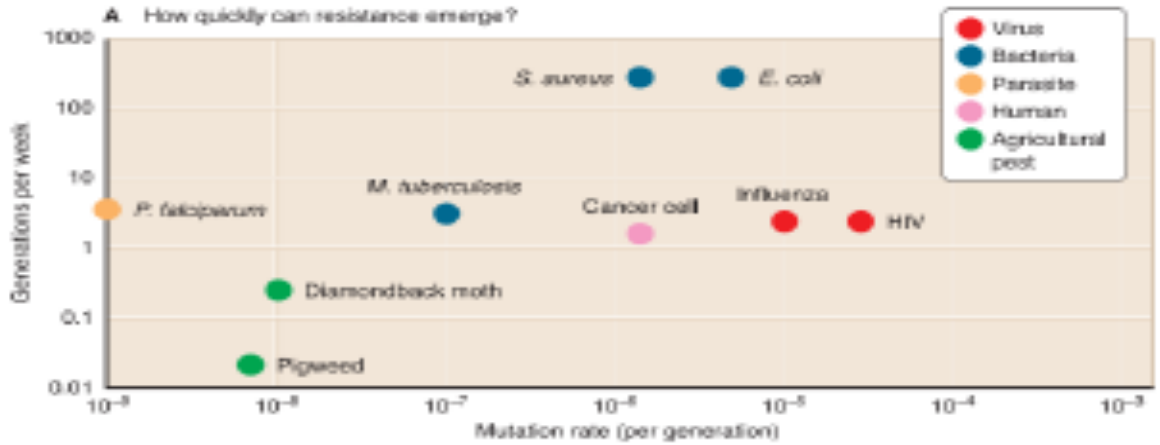


Acoustic Dispensers



Resistance

Application of Drug Combinations to Address Resistance



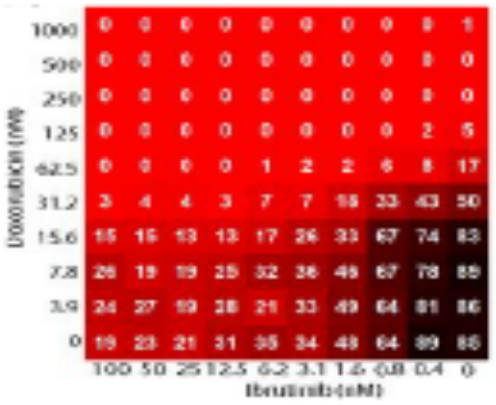
Drug resistance

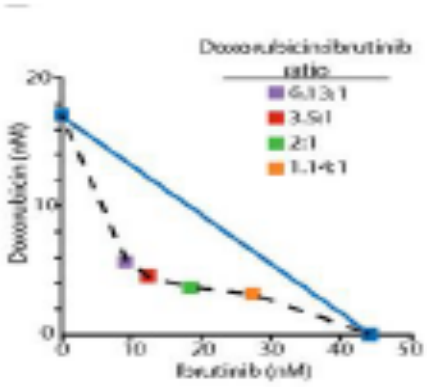
Dissemination of technology: combination screening to overcome drug resistance in cancer cells

- ABC subtype of Diffuse Large B-Cell Lymphoma (ABC-DLBCL) has poor prognosis and response to treatment
- Ibrutinib is a BTK inhibitor that has activity against ABC DLBCL
- Study evaluated 459 drugs in combination with Ibrutinib
  - 6 x 6 concentration-response "matrix blocks", validation in 10 x 10 concentration-response matrix blocks
- DNA-damaging agents identified as synergizing with Ibrutinib in killing ABC DLBCL cell lines
- **Dissemination:**
  - Protocols
  - Source code for dispense

High-throughput combinatorial screening identifies drugs that cooperate with ibrutinib to kill activated B-cell–like diffuse large B-cell lymphoma cells

Lesley A. Mathews Griner[a-1], Rajarshi Guha[a-1], Paul Shinn[a-1], Ryan M. Young[b-1], Jonathan M. Keller[a], Dongbo Liu[c], Ian S. Goldlust[a], Adam Yasgar[a], Crystal McKnight[a], Matthew B. Boxer[a], Damien Y. Duveau[a], Jian-Kang Jiang[a], Sam Michael[a], Tim Mierzwa[a], Wenwei Huang[a], Martin J. Walsh[a], Bryan T. Mott[a], Parseema Patel[a-1], William Leister[a], David J. Maloney[a], Christopher A. Lodish[a], Ganesha Rai[a], Ajit Jadhav[a], Brian D. Peyser[a], Christopher P. Austin[a], Scott E. Martin[a], Anton Simeonov[a], Marc Ferrer[a], Louis M. Staudt[b-1], and Craig J. Thomas[a-1]

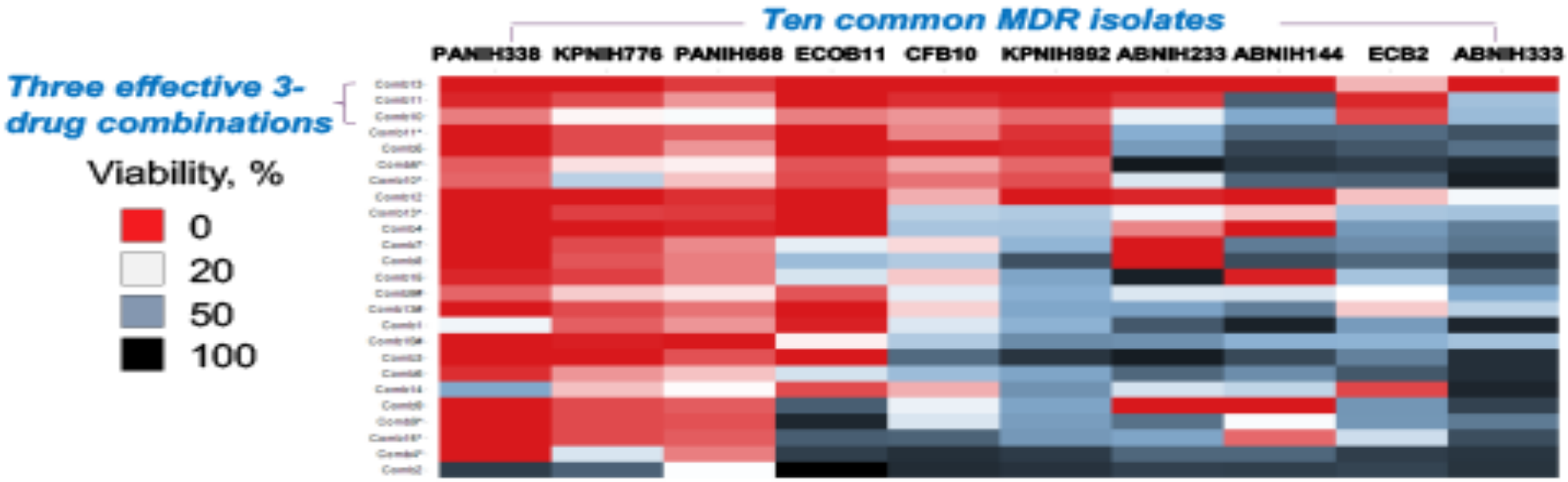




PNAS 111, 2349-2354

Three-drug combinations

Three-drug combinations identified as effective against 10 common MDR clinical isolates

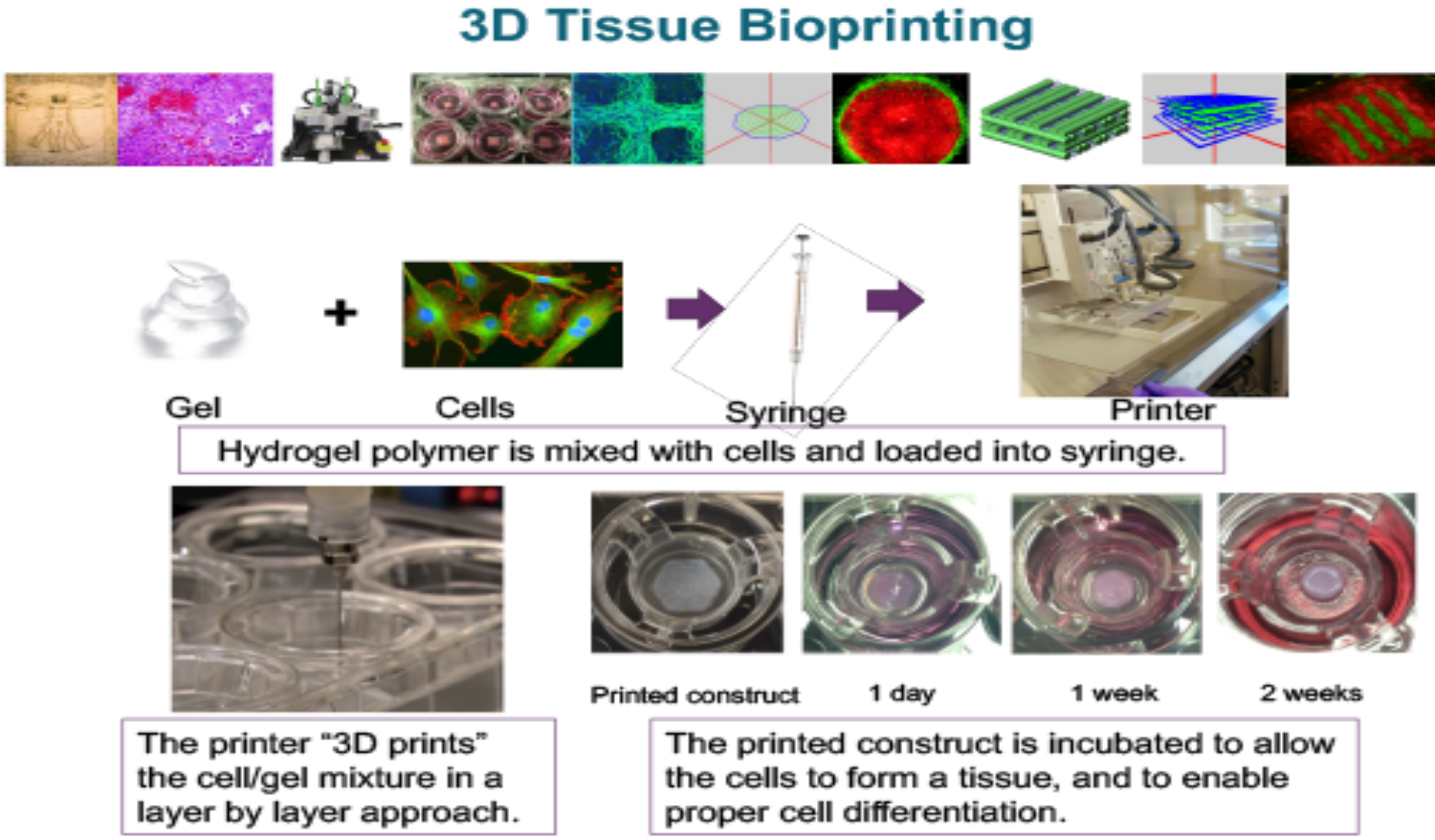


Sun W, et al. Emerging Microbes & Infections ***(2016)***

3D models

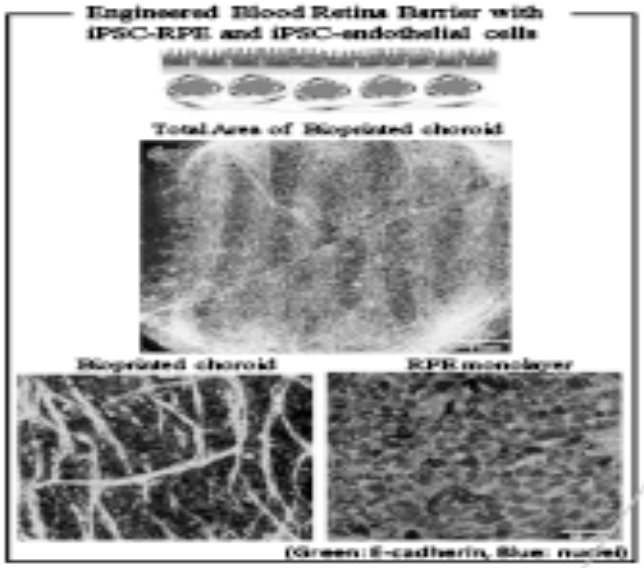

Increasing the predictivity of *in vitro* assays: a continuum of 3D models of healthy and diseased tissues
2D
Spheroids
Organoids
Printed Tissues
Organ-on-a-chip
Physiological complexity
HTS compatibility

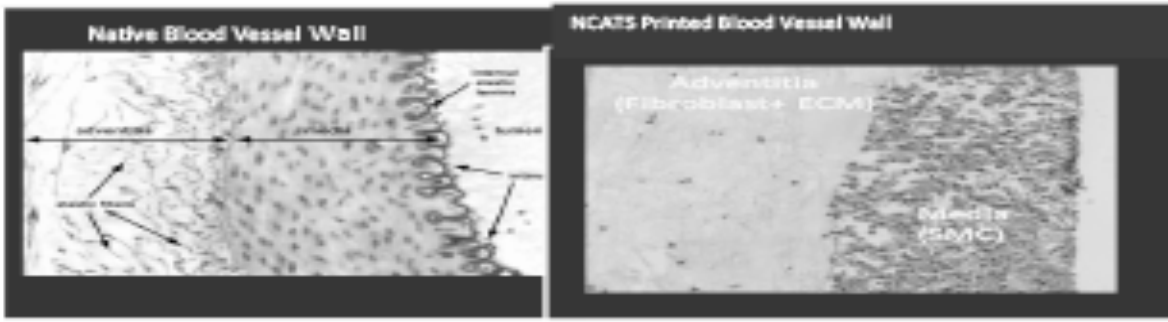
Tissue bioprinting

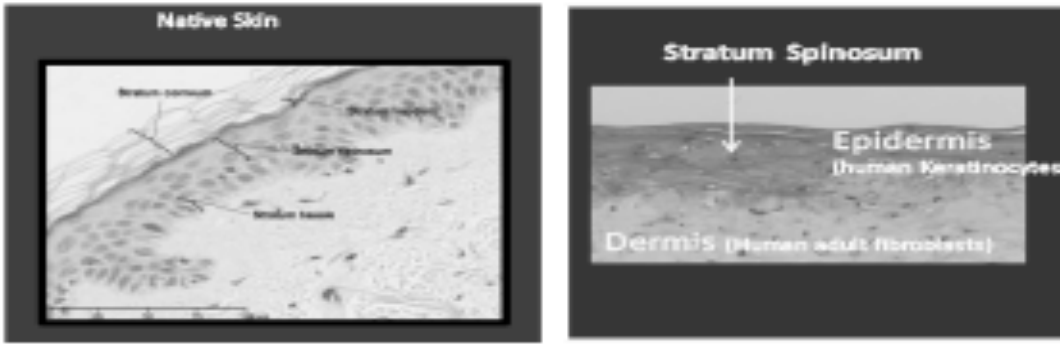


Bioprinting

3D Bioprinting Projects







Blood vessel wall (Kan Cao, U of Maryland)

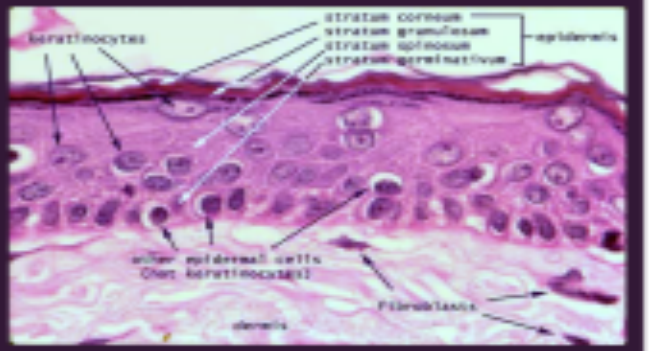
Retina (Kapil Bharti, National Eye Institute)

Skin (Angela Christiano, Columbia University)

Epidermis

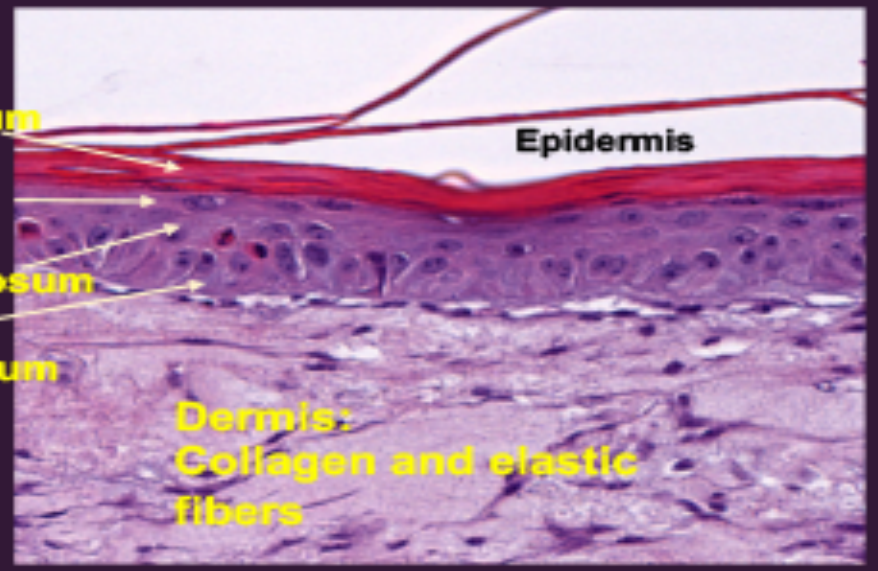
Layers of the Epidermis: native skin *versus* 3D-bioprinted skin

Native Skin



http://www.siumed.edu/~dking2/intro/IN005b.htm

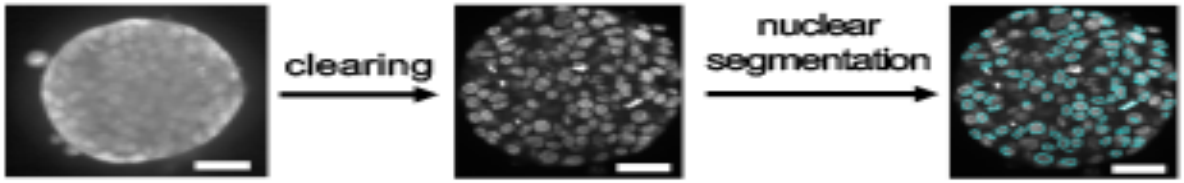
3D-Bioprinted Skin

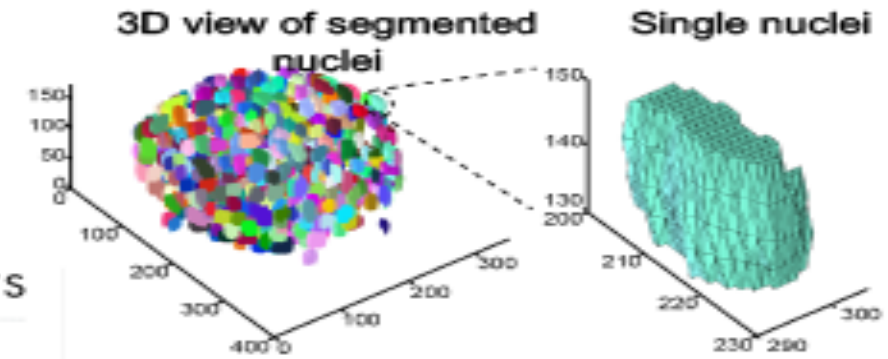


Functional activity analyses

High-throughput tissue clearing protocols for high-content, image and functional activity analyses

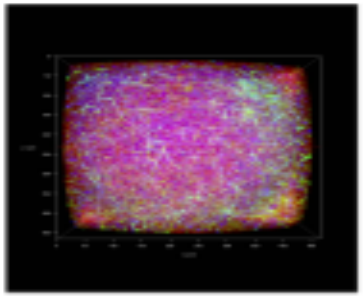
Clearing can be used to improve 3D tissue visualization and analysis





Application of clearing to iPSC-derived neural spheroids

neurons astrocytes nuclei



SCIENTIFIC REPORTS

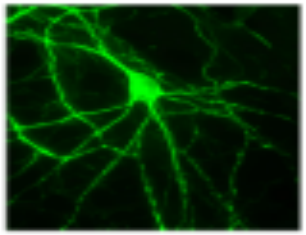
OPEN A high-throughput imaging and nuclear segmentation analysis protocol for cleared 3D culture models

Boutin, *et al*, Sci Rep, 2018

Stem cell technologies

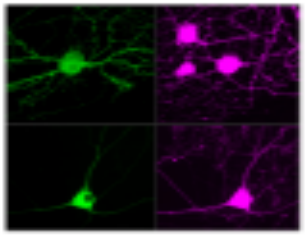
Enabling Advanced 3D Models and Regenerative Medicine through Stem Cell Technologies

NCATS Stem Cell Translation Laboratory:



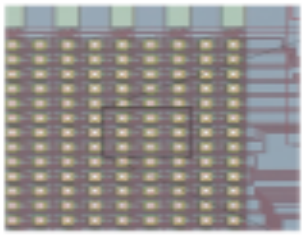
Access to relevant human cell types

Sensory neurons (nociceptors) and other neuronal subtypes, hepatocytes, etc.



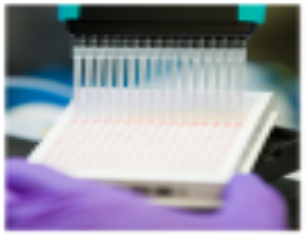
Advanced imaging technologies for functional cell characterization

High-content confocal, calcium imaging, optogenetics



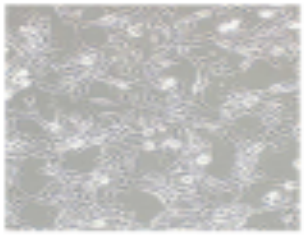
High-throughput electrophysiology methods

High-density multi-electrode arrays 26,400 electrodes/well



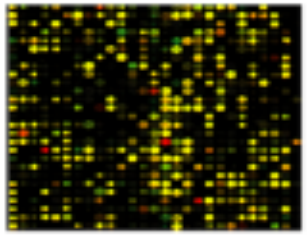
Measurement of signaling pathways, metabolism & specific targets

Cyclic AMP, PKA activity, CREB phosphorylation, energy metabolism



Longitudinal tracking of cell behavior

Multiple measurements over days, weeks or months

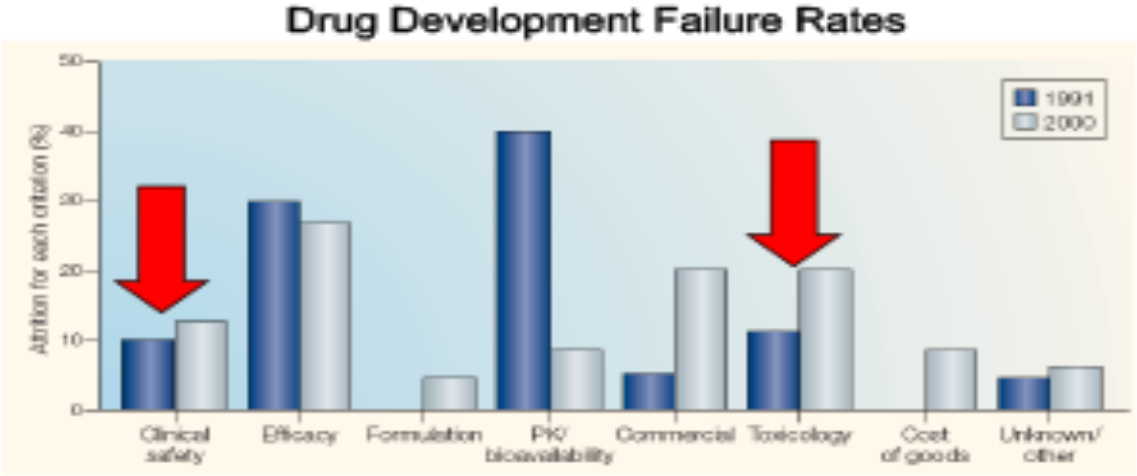


Combined single-cell transcriptomic & proteomic analyses

Drug response in individual cells

Toxicity

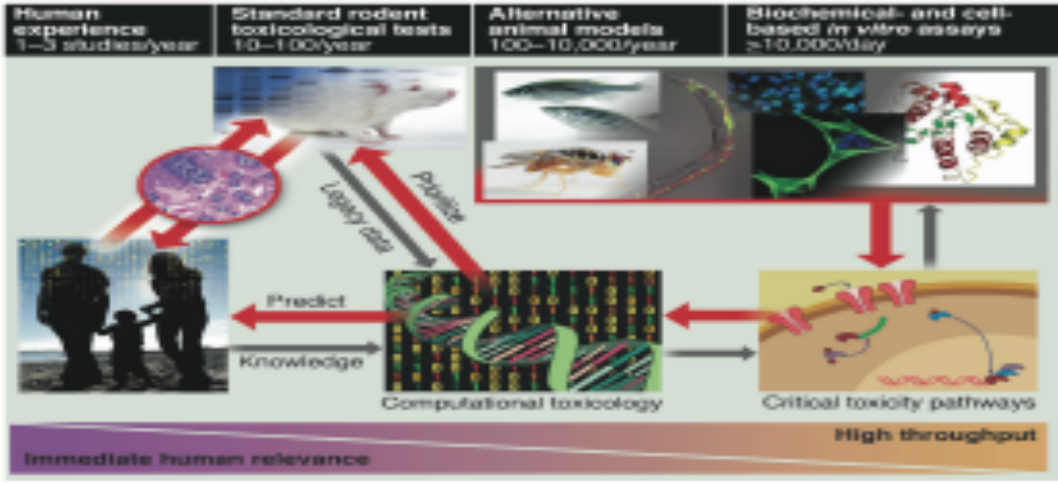
Improved prediction of toxicity is crucial in drug development *and* environmental protection



Preclinical (21%) + Clinical (12%) Tox = 33% of all failures

Kola and Landis, *Nature Reviews Drug Discovery* 3, 711-716, 2004.

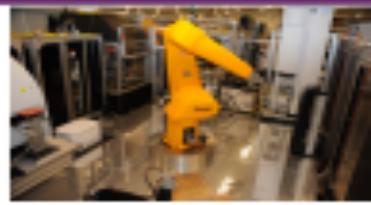
- There are over 80,000 chemicals in commerce, the majority with little to no toxicological data.
- We cannot solve the problem using laboratory animal tests only.



Collins, Gray, Bucher (2008) *Science* 319:906-7

Tox21 screening

The Tox21 Screening Project



Collection of diverse chemicals

In vitro test methods, screening

High quality bioactivity data

Predictive models (of bioactivity of a new chemical *in vitro* and, one day, *in vivo*)

>50 screening campaigns of the 10K Collection

Tox21 10K Chemical Collection: ~10,000 chemicals (nominated and procured by EPA, NIEHS, and NCATS) comprising approved drugs, failed drugs, pesticides, industrial chemicals, etc. Extensive Quality Control →

Screening questions

Tox21 Screening Outcomes

- Rapid testing of chemicals enabled through robotic screening, largest collection of environmental chemicals and drugs assembled, multiple QC measures in place.
- Deposition into the public domain of the largest-ever toxicology dataset (almost 100M datapoints).
- Using crowdsourcing to move from data to knowledge.
- Estrogen receptor *in vitro* data being used by the EPA for regulatory purposes (https://www.epa.gov/endocrine-disruption/use-high-throughput-assays-and-computational-tools-endocrine-disruptor).
- Multiple organizations and consortia worldwide using Tox21 data (*e.g.*, eTox/IMI, Tiley *et al. Environ Int* 101:19-26 used Tox21 data to rank chemicals of concern at Superfund sites).

Information sharing

Sharing internal know-how: Assay Guidance Manual (47 chapters/ 1,338 printed pages)

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https://ncats.nih.gov/agm-video

August 7th Videos

1. Austin, CP: **Welcome to the Assay Guidance Manual (AGM) Workshop**
2. Coussens, NP: **Strategies for Assay Selection & Robust Biochemical Assays**
3. Riss, T: **Treating Cells as Reagents to Design Reproducible Screening Assays**
4. Trask, OJ: **Assay Development Considerations for High Content Imaging**
5. Auld, DS: **Studies in Mechanisms and Methods in Assay Interferences**
6. Dahlin, JL: **Assay Interference by Chemical Reactivity**
7. Chung, TDY: **Basic Assay Statistics, Data Analysis & Rules of Thumb**
8. Devanarayan, V: **Reproducibility & Differentiability of Potency Results**
9. Sittampalam, GS: **Avoiding Artifacts & Interferences in Assay Operations**

March 26-27th Videos

1. Austin, CP: **Welcome to the Assay Guidance Manual (AGM) Workshop**
2. Coussens, NP: **Robust Assays Define Success in Preclinical Research**
3. Lal-Nag, M: **Target Identification & Validation in Translational Discovery**
4. Foley, TL: **Development & Validation of Cell-Based and Biochemical Assays**
5. Riss, T: **Treating Cells as Reagents to Design Reproducible Screening Assays**
6. Trask, OJ: **Assay Development for HCS & Best Practices for 3D HCS**
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14. Weidner, JR: **Assay Operations: Keeping Assays Robust and Reproducible**

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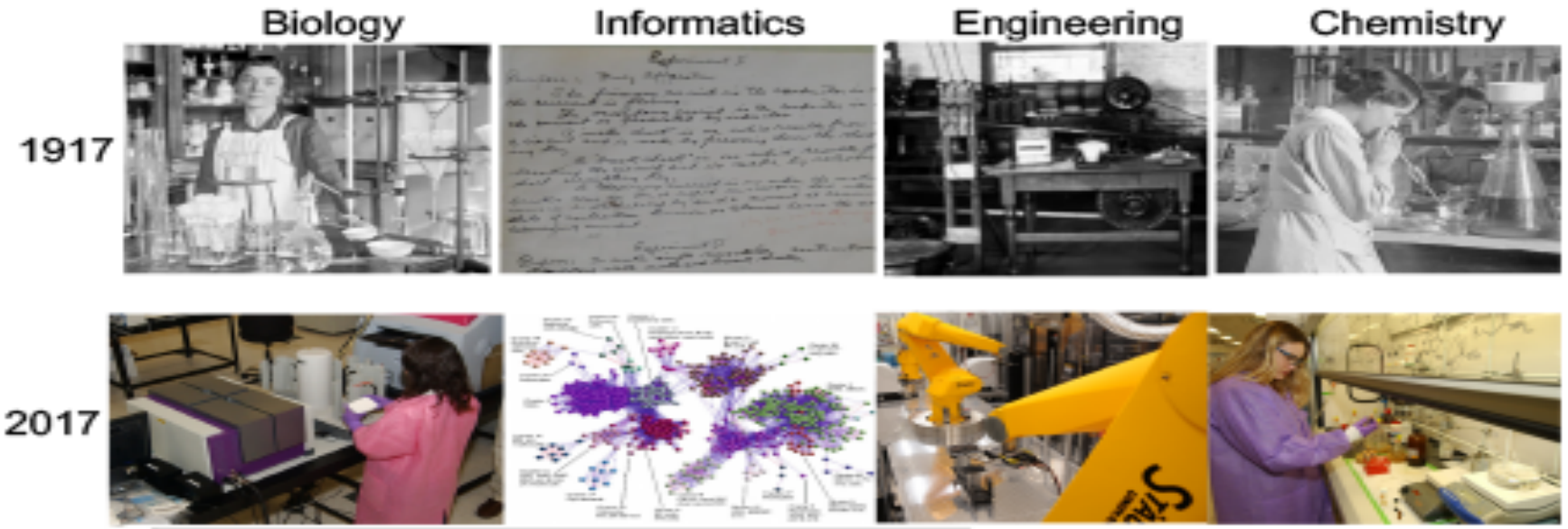
Automating chemistry

Looking Ahead…Automating Chemistry as the Next Challenge in Preclinical Translation

Changes in biomedical research fields in the last 100 years:



Mapping biologically active chemical space to accelerate drug discovery

G. Sitta Sittampalam*, Dobrila D. Rudnicki, Danilo A. Tagle, Anton Simeonov and Christopher P. Austin

A specialized platform for innovative research exploration — ASPIRE — in preclinical drug discovery could help study unexplored biologically active chemical space through integrating automated synthetic chemistry, high-throughput biology and artificial intelligence technologies.

NATURE REVIEWS | DRUG DISCOVERY

doi:10.1038/d41573-018-00007-2

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